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16. ABSTRACT

LC50 values for 30-day old fathead minnows (Pimephales promelas) exposed to bromacil were 185, 183, 182, and 167 mg/L at 24, 48, 96, and 168 hr, respectively; and for exposure to diuron were 23.3, 19.9, 14.2, and 7.7 mg/L at 24, 48, 96, and 192 hr, respectively. In early life-stage exposures, it was not possible to determine a "no effect" concentration for bromacil, as growth was reduced at the lowest exposure of 1.0 mg/L. The "no effect" concentration for diuron was 33.4 µg/L following a 64-day exposure, while a concentration of 78.0 µg/L adversely affected fry in their appearance and survival. It also affected the survival of juvenile fish. Neither herbicide accumulated significantly in fish tissue. Rainbow trout (Salmo gairdneri) injected with radiolabeled bromacil or diuron eliminated over 90% of the radioactivity within 24 hr. Metabolites of diuron included 3,4-dichloroaniline and several demethylated products.

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Bromacil and Diuron Herbicides: Toxicity, Uptake, and Elimination in Freshwater Fish

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Abstract. Fathead minnows, 30 days old, were exposed to technical grade bromacil and diuron in flow-through tests to determine acute toxicity. LC_{50} values for bromacil were 185, 183, 182 and 167 mg/L at 24, 48, 96, and 168 hr, respectively; and for diuron, 23.3, 19.9, 14.2, and 7.7 mg/L at 24, 48, 96, and 192 hr, respectively. Eggs, newly hatched fry, and juvenile fish were continuously exposed to lower concentrations of the herbicides for 64 days. Growth was significantly reduced ($p \leq 0.01$) at the lowest bromacil exposure of 1.0 mg/L. Therefore, it was not possible to determine a "no effect" concentration. The "no effect" concentration for diuron was 33.4 μg/L, while the lowest concentration which resulted in adverse effects was 78.0 μg/L. Adverse effects at 78.0 μg/L were an increased incidence of abnormal or dead fry immediately after hatch ($p \leq 0.01$) and decreased survival throughout the exposure period ($p \leq 0.05$). Neither herbicide accumulated significantly in fish tissue, as bioconcentration factors were <3.2 and 2.0 for bromacil and diuron, respectively. Rainbow trout (*Salmo gairdneri*) injected with radiolabeled bromacil or diuron eliminated over 90% of the radioactivity within 24 hr. Parent compound and metabolites were detected in the aquarium water in both cases. Metabolites of diuron recovered from the water included 3,4-dichloroaniline and several demethylated products.

Bromacil (5-bromo-3-*sec*-butyl-6-methyluracil) is an herbicide primarily used for control of perennial grasses, residual weeds and brush along ditch or canal banks, highways, railroads, utility lines and at industrial sites. (Crafts 1975; US EPA 1974). The herbicide diuron [3-(3,4-dichlorophenyl)-1,1-dimethylurea] has been used for selective control of annual grasses and broadleaf weeds in orchards and vineyards, pineapples, sugarcane, alfalfa and cotton (Crafts 1975; Majka and Lavy 1977). It has also been variously used for aquatic weed control (Johnson and Julin 1974). Bromacil and diuron have been used in combination to provide a broad spectrum of weed control in citrus crops as well as in noncrop situations (Farm Chemicals Handbook 1984).

Bromacil and diuron are two of the more persistent herbicides in soils. They both provide full season weed control (Klingman and Ashton 1975), and they maintain phytotoxicity for up to 24 months (Radosevich *et al.* 1975). Although these herbicides have been marketed for many years, their respective hazards to non-target aquatic organisms have been difficult to assess due to a lack of published studies in the areas of chronic toxicity and bioconcentration. This study was conducted to address these areas, using freshwater fish as test organisms.

Materials and Methods

Test Chemicals

Technical grade bromacil was supplied by DuPont Chemical Co (Chicago, IL) at a purity level of 95.0%. ^{14}C-labeled bromacil was synthesized by New England Nuclear (Boston, MA) and

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provided by DuPont Chemical Co. It was radiolabeled at the number 2 carbon position, and had a specific activity of 6.26 mCi/mmol. Technical grade diuron was received from DuPont Chemical Co with a purity of 98.6%. ^{14}C-labeled diuron was supplied by DuPont Chemical Co as carbonyl ^{14}C-labeled compound with a specific activity of 0.98 mCi/mMol, and by California Bionuclear Corporation (Sun Valley, CA) as uniformly ring-labeled compound with a specific activity of 12.2 mCi/mmol.

Water Characteristics

Lake Superior water was used in fish culture and in test exposure systems. Mean values (±s.d.) for total hardness, total alkalinity, acidity (as mg/L $CaCO_3$) and pH were 47.4 ± 2.8, 43.0 ± 2.3, 2.2 ± 0.6, and 7.4 ± 0.1, respectively (n = 18–28).

Toxicity Tests

Acute flow-through tests were conducted with technical grade bromacil and diuron by methods described previously (Call *et al.* 1983, 1984). Due to the low level of acute toxicity for bromacil, a stock solution was prepared by dissolving bromacil to a near-saturation level in a 230 L reservoir of Lake Superior water. A stock solution of diuron was generated from a sand column, similar to the procedure of Veith and Comstock (1975).

Bromacil concentrations in water were measured by spectrophotometry at 280 nm. Mean bromacil exposures over the 8-day acute test were 108 ± 7.4, 127 ± 8.8, 146 ± 7.8, 172 ± 9.0 and 207 ± 11 mg/L (n = 9). Diuron concentrations in the acute test were measured by spectrophotometry at 250 nm. Mean diuron exposures were 5.54 ± 0.47, 7.94 ± 0.43, 11.1 ± 0.88, 16.4 ± 0.76, and 24.2 ± 0.23 mg/L (n = 4–12).

The mean water temperature for the bromacil acute test was 25.0 ± 0.4°C (range = 24.1–25.9°C, n = 96), and for the diuron test was 24.3 ± 0.8°C (range = 22.3–25.6°C, n = 94). Dissolved oxygen levels were maintained between 95.5 and 96.6% of saturation for the bromacil test (n = 9) and between 88.6 and 94.5% for the diuron test (n = 4).

Early life-stage (ELS) exposures were initiated with fertilized eggs <24 hr old. From 92 to 108 eggs were added to the duplicate incubation cups in each diluter exposure chamber. Thirty fry were transferred into each aquarium containing 4.5 L of water upon completion of hatch (4–5 days), and the fry and juveniles were continuously exposed to the herbicides for 60 days. Fish were fed finely granulated dry fish food (Tetramin®) and newly hatched brine shrimp for the first month and frozen adult brine shrimp during the second month. Equal volumes of food were provided to all aquaria.

Mean bromacil concentrations (measured as in acute test) were 1.0 ± 0.4, 1.9 ± 0.2, 4.4 ± 0.5, 12.0 ± 1.0, and 29.0 ± 2.1 mg/L (n = 33–36). Mean diuron exposures were 2.6 ± 0.7, 6.1 ± 1.6, 14.5 ± 2.0, 33.4 ± 4.8, and 78.0 ± 8.1 µg/L (n = 36–38). Diuron concentrations in the ELS test were determined by extraction with methylene chloride, evaporation of the solvent to dryness, dissolution of the residue in methanol, and analysis by high pressure liquid chromatography (HPLC), based upon the procedure of Farrington *et al.* (1977). A Waters Associates Model 6000A instrument was used with a Model 440 detector at a fixed wavelength of 254 nm. A Porasil®-18 column was operated at ambient temperature. The mobile phase of 60% methanol and 40% water had a flow rate of 2 mL/min, and the column was operated at a pressure of approximately 3800–4000 psi.

Exposure chambers received 0.5 L of water plus toxicant every 8–11 min. The mean water temperature for the bromacil ELS test was 24.4 ± 2.6°C (n = 324), with a range from 22.9 to 27.2°C; and for the diuron ELS test the mean water temperature was 25.0 ± 0.7°C (n = 254), with a range from 23.8 to 27.0°C. Mean dissolved oxygen in the bromacil test was 86.8 ± 5.4% of saturation (n = 13, range = 77.0–92.5%) for control and exposure aquaria combined, while in the diuron test it was 91.2 ± 1.5% of saturation (n = 9, range = 88.3–92.9%).

Early life-stage test endpoints included egg hatchability, deaths or abnormalities in newly hatched fry, survival of juvenile fish throughout the exposure period, and fish growth. Data were analyzed by one-way analysis of variance in conjunction with Dunnett's Procedure (Steel and Torrie 1960).

Tissue Accumulation and Elimination

Solutions of ^{14}C-labeled bromacil and diuron in methanol were delivered to aquaria containing one hundred 30-day old fathead minnows. All test aquaria, including controls, received 100 mg/L methanol. Mean water temperature was 24.2 ± 0.9°C (range = 22.9–25.5°C) in the bromacil study, and 24.8 ± 0.4°C (range = 24.1–25.8°C) in the diuron study. Fish were exposed to two concentrations of each herbicide which differed by approximately an order of magnitude. For both test chemicals, water samples were extracted with methylene chloride, the solvent evaporated to dryness, and the residue dissolved in 5 mL toluene. Scintillation fluid (15 mL) was added, and the radioactivity measured as described (Call *et al.* 1984). Mean (±s.d.) bromacil concentrations were 4.25 ± 0.46 and 35.1 ± 4.00 µg/L (n = 7) over a 17-day exposure period. Mean (±s.d.) diuron concentrations were 3.15 ± 0.61 and 30.4 ± 4.36 µg/L (n = 9) over a 24-day exposure. The fish were fed daily equal volumes of frozen adult brine shrimp to all aquaria. Five fish were removed and individually analyzed for total ^{14}C as previously described (Call *et al.* 1984) on days 1, 2, 4, 7, 10, 14, and 17 in the bromacil study, and at the same intervals plus days 21 and 24 in the diuron study.

In the diuron study, several fish were frozen at the end of the exposure period for the determination of relative amounts of parent herbicide and metabolites. Fish were not saved for this purpose in the bromacil study, due to very low levels of total ^{14}C present in the fish.

At the end of each exposure period, the remaining fish were transferred to a 27-L aquarium which received only Lake Superior water at the rate of 4 L/hr. Elimination of ^{14}C was determined by individually analyzing samples of five fish at selected intervals over a period of 17–21 days.

In Vivo Metabolism Studies

Samples of fathead minnows exposed to radiolabeled diuron were collected at the end of the uptake phase and homogenized with 6.5 mL of water in a Potter-Elvehjem homogenizer. A 50 µl aliquot of the homogenate was analyzed for total ^{14}C radioactivity. The homogenate was acidified with 0.4 mL concentrated HCl, and extracted three times with ethyl ether. Ether extraction failed to remove any radioactivity from the aqueous phase.

The aqueous layer was centrifuged and the resulting pellet was

oxidized in a Packard Model 306 sample oxidizer. The trapped $^{14}CO_2$ was counted in a liquid scintillation spectrometer to determine particulate-bound ^{14}C. The supernatant was lyophilized and the residue dissolved in a small volume of acetone for thin-layer chromatography (TLC). Solutions of the water-soluble fraction in acetone were applied to silica gel TLC strips and co-chromatographed with a diuron standard. TLC strips were developed with 90/10 (v/v) toluene/methanol.

Developed TLC strips were first analyzed for ^{14}C activity on a radio-chromatogram scanner. Radioactive bands were scraped from the strips, and compounds eluted from the silica gel with methanol. Eluates were added to scintillation fluid and counted to determine relative contributions of parent herbicides and metabolites toward total ^{14}C in the water-soluble fraction.

Rainbow trout (*Salmo gairdneri*) were used in herbicide metabolism studies due to the small size of the fathead minnows. Trout weighing 100–150 g were injected with 1 μCi of ^{14}C-labeled bromacil or diuron. With diuron, injection of the radiolabeled herbicide was also performed on trout which had been pre-exposed to unlabeled herbicide at a sublethal concentration for 4–5 days. Individual trout were held unfed in a shaded 10–12L aquarium maintained at 10°C. Fish were sacrificed 24 hr after injection.

Aquarium water containing bromacil or diuron metabolites was passed through a column (2.5 × 30 cm) of Amberlite® XAD-2 resin. The column was washed with 1–2 bed volumes of distilled water, then eluted with 900 mL of methanol. Aliquots of the water prior to XAD-2 treatment and of the methanol eluate were counted in a Packard Tricarb liquid scintillation counter to determine total radioactivity recovered from the column. The methanol eluate was evaporated to dryness in a stream of air prior to TLC.

Trout liver was homogenized with 20–25 volumes of cold acetone in a Waring blender, followed by centrifugation for 5 min at 900 × g to remove insoluble solids. The acetone extract was dried with sodium sulfate and then evaporated in a stream of air. An aliquot of the acetone extract was counted in a scintillation counter and the solid residue processed in a Packard Sample Oxidizer to determine unextracted radioactivity.

Bile was diluted 1:5 with water, acidified with HCl, and extracted three times with 10 mL of ethyl ether. The ether extract was evaporated to dryness in a stream of nitrogen, and the residue redissolved in acetone prior to TLC. Thin-layer chromatograms were examined under ultra-violet light for absorbing spots or bands, and radioactive areas located with a Packard radiochromatogram scanner. Areas which were both radioactive and ultra-violet absorbing were scraped to remove the silica gel, and the silica gel eluted with methanol. The eluted material was analyzed by gas chromatography/mass spectrometry (GC/MS) as previously described (Call *et al.* 1983).

Gas Chromatography-Mass Spectrometry (GC/MS)

Exposure water and fish bile were analyzed for herbicide metabolites by GC/MS. The technical grade diuron was also analyzed by GC/MS for the presence of 3,3',4,4'-tetrachloroazobenzene and 3,3',4,4'-tetrachloroazoxybenzene, impurities that have been associated with diuron synthesis (Hill *et al.* 1981; Sündstrom *et al.* 1978). Specific techniques were described earlier (Call *et al.* 1983).

Results and Discussion

Acute Toxicity

Mean LC_{50}'s for pooled replicates of bromacil at 24, 48, 96, and 168 hr were 185, 183, 182 and 167 mg/L, respectively. Fathead minnows were similar in sensitivity to carp (*Cyprinus carpio*) with 24 and 48 hr LC_{50}'s of 164 mg/L, but less sensitive than bluegill sunfish (*Lepomis macrochirus*) or rainbow trout, with 48 hr LC_{50}'s of 71 and 75 mg/L, respectively (US EPA 1975).

LC_{50}'s for pooled diuron replicates at 24, 48, 96, and 192 hr were 23.3, 19.9, 14.2, and 7.7 mg/L, respectively. In a review of diuron (Johnson and Julin 1974), the 96 hr LC_{50}'s for fish generally ranged from 1 to 25 mg/L. Larval striped bass (*Morone saxatilis*) were most sensitive with a 96 hr LC_{50} of 0.5 mg/L (Hughes 1973). Largemouth bass (*Micropterus salmoides*) were resistant to diuron at its solubility limit of 42 mg/L (Bond *et al.* 1960).

Early Life-Stage Toxicity

Bromacil exposures did not significantly affect ($p \leq 0.05$) egg hatch, percent of newly hatched fry that were grossly abnormal in appearance or that died immediately upon hatch, or survival of fish through 60 days post-hatch (Table 1). All exposures resulted in a reduction ($p \leq 0.05$) in wet weight. With the exception of the bromacil exposure of 1.9 mg/L, all treatments also resulted in reductions in length ($p \leq 0.05$).

Due to significant growth reduction at the lowest bromacil exposure of 1.0 mg/L, a "no effect" concentration was not determined. The glass diluter tubes leading to bromacil treatment aquaria were much cleaner than the tubes leading to control aquaria, and this was attributed to algacidal properties of bromacil. Bromacil at 2 mg/L markedly inhibited photosynthesis by a wild strain of Euglena (Hoffman 1971). Growth reductions in bromacil-exposed fish in the present study may have been due, in part, to reductions in population densities of algae in the aquaria, rather than solely to bromacil toxicity; young fathead minnows feed upon algae under natural conditions (Eddy and Underhill 1976).

Bromacil residues in forest streams have been found 1.5 yr following application at concentrations of approximately 0.25 mg/L (Pierce 1969). However, it is unlikely that concentrations which are acutely toxic to fish would be encountered in

Table 1. Hatchability, development, survival and growth of fathead minnows (*Pimephales promelas*) exposed to bromacil for 64 days

Parameter	Mean bromacil concentration ± s.d. (mg/L)					
	0.0 ± 0.0 (n = 34)	1.0 ± 0.4 (n = 36)	1.9 ± 0.2 (n = 36)	4.4 ± 0.5 (n = 36)	12.0 ± 1.0 (n = 33)	29.0 ± 2.1 (n = 36)
Mean % hatch[a]	66.0	68.8	58.4	58.5	60.4	72.3
Mean % abnormal and dead[b]	5.9	6.9	7.4	8.3	8.4	11.5
Mean number of survivors at 60 days post-hatch[c]	26.5	28.5	29.5	29.5	27.5	26.5
Mean wet weight at 60 days post-hatch (g)	0.479	0.410**	0.420*	0.381**	0.374**	0.326**
Mean standard length at 60 days post-hatch (mm)	29.8	28.4*	28.7	27.9**	27.7**	26.4**

[a] Live fry/total eggs after five days
[b] [Abnormal (deformed) + dead fry]/total fry at time of transfer from egg cups (5 days after initial exposure of eggs)
[c] Based on 30 fry transferred to duplicate exposure chambers
* Significantly different from controls ($p \leq 0.05$)
** Significantly different from controls ($p \leq 0.01$)

Table 2. Hatchability, development, survival and growth of fathead minnows (*Pimephales promelas*) exposed to diuron for 64 days

Parameter	Mean diuron concentration ± s.d. (µg/L)					
	0.0 ± 0.0 (n = 18)	2.6 ± 0.7 (n = 38)	6.1 ± 1.6 (n = 36)	14.5 ± 2.0 (n = 37)	33.4 ± 4.8 (n = 38)	78.0 ± 8.1 (n = 38)
Mean % hatch[a]	67.9	77.9	75.0	71.8	67.9	66.1
Mean % abnormal and dead[b]	2.2	0.6	1.3	3.7	8.2	15.0**
Mean number of survivors at 60 days post-hatch[c]	24.5	26.5	28.0	21.5	22.5	7.5*
Mean wet weight at 60 days post-hatch (g)	0.568	0.568	0.563	0.619	0.563	0.496
Mean total length at 60 days post-hatch (mm)	32.2	32.3	32.4	32.3	31.0	29.1

[a] Live fry/total eggs after 5 days
[b] [Abnormal (deformed) + dead fry]/total fry at time of transfer from egg cups (5 days after initial exposure of eggs)
[c] Based on 30 fry transferred to duplicate exposure chambers
* Significantly different from controls ($p \leq 0.05$)
** Significantly different from controls ($p \leq 0.01$)

streams or lakes following normal recommended rates of application. Additional monitoring data would be useful in determining whether residues might affect growth of fish or other aquatic animals, either directly or indirectly via their food chains.

Diuron exposures ranging from 2.6 to 78 µg/L did not significantly affect ($p \leq 0.05$) egg hatch or fish growth (wet weight and length) at 60 days post-hatch (Table 2). However, at the highest exposure of 78 µg/L, the percentage (15.0%) of newly hatched fry that died or were grossly deformed was significantly greater ($p \leq 0.01$) than in the control group (2.2%). Survival of fish over the 60 day exposure was also reduced ($p \leq 0.05$) at this concentration. The maximum acceptable toxicant concentration (MATC) was determined to lie between 33.4 and 78.0 µg/L.

The toxic impurities, 3,3′,4,4′-tetrachloroazobenzene (TCAB) and 3,3′,4,4′-tetrachloroazoxybenzene (TCAOB), which have been reported to be present in some diuron formulations, were not detected. Limits of detection for TCAB and TCAOB were 10 and 30 µg/g, respectively. Thus, the early life-stage toxicity observed cannot be attributed to these impurities. In an early life-stage toxicity test with propanil (Call *et al.* 1983), 670 µg/g TCAB was measured in the technical grade product; TCAB may have contributed to the high level of toxicity.

In a Hawaiian watershed in which diuron was used extensively for weed control in pineapple and sugarcane fields, diuron concentrations in field runoff ranged from 3 to 32 µg/L, while concentrations in estuarine waters ranged from 0.1 to 1.0 µg/L (Green *et al.* 1977). Diuron was considered to

have been mainly transported from the fields to the streams in solution rather than by soil particle erosion. By contrast, on nearly level land in the lower Mississippi River Valley where diuron was applied to plots of cotton, diuron was either not detectable or present only in trace amounts (<10 μg/L) in runoff (Willis *et al.* 1975). Stream concentrations would have been even less, due to sediment deposition and dilution. In a study in which experimental outdoor ponds were treated with 0.5 to 3.0 mg/L diuron, (McCraren *et al.* 1969), residues in the water ranged from 25 to 70 μg/L after 7 days. Residues from 10 to 26 μg/L were measured after 14 days in ponds treated with 1.5 and 3.0 mg/L diuron. A residue in the water of 35 μg/L was measured after 21 days in a 3.0 mg/L pond, but no residues were measured at 28 days. Diuron persisted in the mud for over 100 days in all treated ponds at concentrations ranging from 0.01 to 0.10 mg/kg.

Apparently, from these residue studies and our early life-stage toxicity study, concentrations of diuron present in bodies of water as a result of applications for field crop weed control would not be great enough to directly affect survival and growth of fish or other aquatic animals having sensitivities to diuron similar to that of fathead minnows. However, indirect effects due to reduced plant growth might occur, as diuron reduced the biomass of the freshwater alga *Scenedesmus quadricaudata* by 99% at 100 μg/L (Stadnyk *et al.* 1971), and prevented growth of some marine phytoplankton species at concentrations as low as 0.02 μg/L (Ukeles 1962). If treated lakes or ponds contained residues for several weeks of the magnitude observed by McCraren *et al.* (1969), mortalities would be likely to occur among the more sensitive fish and invertebrate species.

Uptake and Elimination

Fathead minnows exposed to ^{14}C-bromacil for 17 days bioconcentrated the radiolabel in whole fish to a mean level of only 3.2 times the concentration in water. Due to the very low amount of radioactivity in the fish, metabolite characterization was not pursued.

^{14}C-diuron was rapidly taken up by the fish, with an equilibrium between water and fish established within 24 hr (Figure 1). ^{14}C-diuron equivalents were concentrated in fish tissue 157 and 144 times the concentrations in water at lower and higher exposures, respectively. At the end of the 24-day exposure, 15.0% of the total ^{14}C was ether extractable,

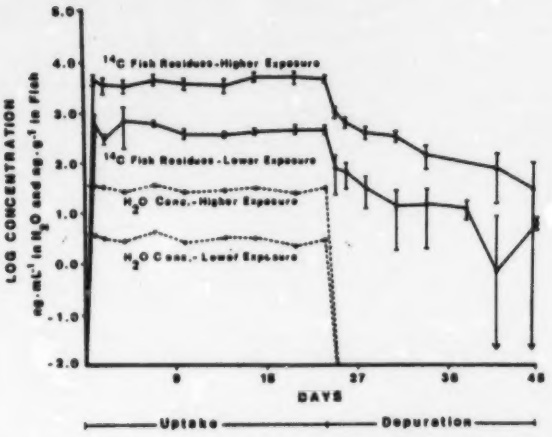

Fig. 1. Log mean exposure water concentration of ^{14}C-labeled diuron (ng · mL^{-1}) and log mean (±S.D.) whole fish total ^{14}C residues (ng · g^{-1}) during uptake and depuration phases

of which 8.4% chromatographed as the parent compound. This constituted 1.3% of the total tissue ^{14}C. From this, a mean bioconcentration factor of 2.0 was determined for diuron.

Diuron was also rapidly eliminated, with 84 and 76% of the radiolabel eliminated within 24 hr after transfer to clean water in lower and higher exposures, respectively. After 21 days in clean water, 98.7 and 99.0% of the ^{14}C had been eliminated at lower and higher exposures, respectively.

These low levels of tissue accumulation and rapid elimination support the statement by Brown (1978) that organic herbicides do not usually accumulate in fish to cause a residue problem, and that residues are quite rapidly lost. Residue accumulation was negligible in fish exposed to simazine (Rodgers 1970), atrazine (Macek *et al.* 1976), the dimethylamine salt of 2,4-D (Sikka *et al.* 1977), and propanil, alachlor, or dinoseb (Call *et al.* 1983, 1984).

However, several herbicides concentrate in fish tissue to a limited extent. Residues of thiobencarb were from 48 to 471 times greater in fish than in water (Sanders and Hunn 1982). Thiobencarb was present in fish sampled during the summer months from a river receiving rice paddy drainage, but was not detectable two months later (Watanabe *et al.* 1983). Residues of chlomethoxynil and molinate were not detected in fish tissue at any time; however, chloronitrofen residues were present in July and September. Pentachlorophenol bioconcentrated in whole fish from about 160 to 1,100 times (Huckins and Petty 1983; Niimi and McFadden 1982; Spehar *et al.* 1985; Veith *et al.* 1979), and tri-

fluralin bioconcentrated approximately 1,000 times (Macek *et al.* 1976).

Metabolism

Radiolabeled bromacil was readily eliminated by trout. In three trout injected with 1.0 μCi ^{14}C-bromacil, over 95% of the ^{14}C appeared in the water within 24 hr. The remainder was in the bile with traces in the blood and liver. Of the radiolabeled material in the water, 70–80% was the parent compound as determined by TLC and GC/MS. The remainder consisted of two or more polar metabolites, which were not characterized.

Radiolabeled diuron was also rapidly eliminated by trout which had received a 1 μCi injection. Over 90% of the radioactivity was present in the water after 24 hr. Parent diuron represented 35–40% of the total radioactivity in the water, the remainder being comprised of at least four other components. Three metabolites in the water were submitted for analysis by GC/MS. The GC oven pyrolysis product study by Büchert and Lokke (1975) was used to interpret the mass spectra. One metabolite was identified as 3,4-dichloroaniline. The spectra of the other two metabolites suggested that they were demethylated compounds, but they were not positively identified.

In man, diuron ingestion produced urinary metabolites identified as 1-(3,4-dichlorophenyl)-3-methyl urea, 1-(3,4-dichlorophenyl) urea, and 3,4-dichloroaniline (Geldmacher *et al.* 1971). Bohme and Ernst (1965) found N-(3,4-dichlorophenyl) urea and N-(2-hydroxy-4,5-dichlorophenyl) urea to be the predominant urinary metabolites in rats fed diuron. Dogs fed diuron on a chronic basis produced demethylated urinary metabolites of N-(3,4-dichlorophenyl) urea and N-(3,4-dichlorophenyl)-N′-methylurea, as well as some 3,4-dichloroaniline and 3,4-dichlorophenol (Hodge *et al.* 1967). No metabolism data are available on aquatic animals.

Summary

This study has shown that bromacil and diuron do not accumulate in fish tissue to a large extent. The low levels of diuron residues in fish tissue were rapidly eliminated upon transfer of the fish to clean water. Neither herbicide was highly toxic to fish upon acute exposure. Potential adverse effects upon fish from application of these herbicides would be more likely to occur with diuron in situations where it was applied directly to the water during a period of embryonic or early post-embryonic development.

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